Molecular identification of the strains contained in commercial products for improving agriculture in Africa

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1. Introduction

There is a proliferation of products appearing on the market that claim major impact in increasing crop productivity. However, most of them have proved to be ineffective in both greenhouse and field conditions and no or inadequate data is available to assess their real effect. These products demand rigorous testing to verify whether they fulfill the claims of the manufacturer. The proliferation of the underperforming products can therefore be prevented, and value can be added to effective products. TSBF-CIAT has been mandated by Bill and Melinda Gate Foundation to scientifically evaluate and select effective commercial products for improving and sustaining crop yields in selected agro ecological zones in Kenya, Nigeria, Niger and Ethiopia.

2. Overall objective

To assess the bacterial content of various commercial agricultural products in comparison to the information given by the private companies.

3. Materials and Methods

The bacterial strains contained in the commercial products were isolated on 2 media: NA (Nutrient Agar) which is a non selective media and allows the growth of most microorganisms and YEMA (Yeast Extract Mannitol Agar) which is a slightly selective media, and allows the growth of Rhizobium. Identification of the strains was done following DNA extraction from liquid cultures and PCR amplification of the 16S rDNA using universal primers (27f and 1492r). The PCR products (rDNA 16S amplified fragments) were submitted for sequencing at SegoliP Unit and the sequence data compared to gene libraries (Genbank) with BLAST (Basic Local Alignment Search Tool) (Altschul *et al.,* 1990) program.

About 100 strains were isolated from 27 products that are classified into 3 categories as follows:

CATEGORY	STRAINS	MODE OF FUNCTION
I	Rhizobial inoculants e.g. *Bradyrhizobium sp., Mesorhizobium sp.,..*	Biological Nitrogen Fixation
IIA	Non-rhizobial bacteria e.g *Azospirillum sp.*	Free living nitrogen-fixing bacteria
IIB	Other micro-organisms e.g. *Bacillus sp.*	Disease suppression, production of metabolites which promote general plant growth or aid in acquisition of less mobile nutrients (particularly P and Zn).

4. Results

Product	Category	Producer	Expected strains	No. of expected strains	No. of isolated strains	SEQUENCING	
						Confirmed as expected strains	Other strains
Chick Pea Nodulator	I	Becker Underwood (USA)	*Mesorhizobium ciceri*	1	1	*Mesorhizobium ciceri*, 98%	-
Legumefix	I	Legume Technology (UK)	*Rhizobium spp.*	1	1	*Bradyrhizobium sp.*, 95%	-
Peanutflo	I	SoyGro (South Africa)	*Bradyrhizobium sp.*	1	5	*Bradyrhizobium yuanmingense*, 98%	*Moraxella sp.*, 99%, *Bacillus sp.*, 97%, *
Soyflo	I	SoyGro Ltd. (South Africa)	*Bradyrhizobium japonicum* (strain WB74)	1	1	*Bradyrhizobium japonicum*, 95%	-
Vault LVL	I	Becker Underwood (USA)	*Bradyrhizobium japonicum*	1	3	*Bradyrhizobium japonicum*, 96%	*Lysinibacillus sphaericus*, 97%, *Bacillus nealsonii*, 98%
Bio N	IIA	Nutri-Tech Solutions (Australia)	*Azotobacter spp.*	1	7	-	*Bacillus megaterium*, 98%, *Bacillus licheniformis*, 98%, *Bacillus firmus*, 99%, *Brevibacillus brevis*, 99%, *
Bioplex	IIA	Nutri-Tech Solutions (Australia)	*Azotobacter spp.*	1	5	-	*Bacillus licheniformis*, 98%, *Stenotrophomonas sp.*, 80%, *2 Bacillus spp.*, 98%, *
Leguspirflo	IIA	SoyGro (South Africa)	*Azospirillum brasilense*	1	4	*Azospirillum brasilense*, 94%	*Bacillus sp.*, 97%, *Paenibacillus polymyxa*, 97%, *Pantoea agglomerans*, 97%
Mazospirflo 1	IIA	SoyGro (South Africa)	*Azospirillum brasilense* CD	1	3	*Azospirillum brasilense*, 98%	*
Mazospirflo 2	IIA	SoyGro (South Africa)	*Azospirillum brasilense* CD	1	1	*Azospirillum brasilense*, 97%	-
Rhizostim	IIA	SoyGro (South Africa)	*Azospirillum* sp.	1	1	-	*Bacillus sp.*, 97%
Twin-N	IIA	Mapleton (UK)	*Azorhizobium sp., Azoarcus sp., Azospirillum sp.*	3	2	*Azorhizobium caulinodans*, 98%, *Azospirillum brasilense*, 98%	-
Integral	IIB	Becker Underwood (USA)	*Bacillus subtilis* (strain MBI 600)	1	1	*Bacillus subtilis*, 97%	-
Subtilex	IIB	Becker Underwood (USA)	*Bacillus subtilis* (strain MBI 600)	1	1	*Bacillus sp.*, 96%**	-
Bac up	IIB	Biological Control Products (South Africa)	*Bacillus subtilis*	1	2	*Bacillus sp.*, 98%**	*Paenibacillus sp.*, 98%
Defender	IIB	Madumbi (South Africa)	*Bacillus subtilis*	1	2	*Bacillus sp.*, 98%**	*Brevibacillus borstelensis*, 97%
NIB PGPR peat inoculant	IIB	Murdoch University (Australia)	*Pseudomonas sp.*	1	1	*Pseudomonas fluorescens*, 95%	-
MycoApply MAXX	IIB	Mycorhizal Applications (USA)	*Bacillus licheniformis, B.pumilis, B.amyloliquefaciens, B.megaterium*	4	13	*Bacillus megaterium*, 97%, 5 *Bacillus spp.*, 97%**	*Paenibacillus lactis*, 97%, *Bacillus cereus*, 97%, *Brevibacillus borstelensis*, 98%, *Bacillus subtilis*, 98%, *Paenibacillus polymyxa*, 96%, *Enterobacter pulveris*, 97%, *
Myco Apply Soluble Maxx	IIB	Mycorhizal Applications (USA)	*Bacillus licheniformis, B.pumilis, B. azotoformans, B. megaterium, B. coagulans, B. thuringiensis, B. stearothermiphilis, Paenibacillus polymyxa, P. durum, P. Gordonae, Azotobacter polymyxa, A. chroococcum, Pseudomonas fluorescens, P. aureofaceans*	15	7	4 *Bacillus spp.*, 98% **	*Bacillus subtilis*, 97%, *Bacillus circulans*, 100%, *Enterobacter pulveris*, 97%
B.Sub	IIB	Nutri-Tech Solutions (Australia)	*Bacillus subtilis*	1	4	*Bacillus subtilis*, 98%	*Brevibacillus brevis*, 96%, *Bacillus cereus*, 91%, *
Accelerate	IIB	Nutri-Tech Solutions (Australia)	*Bacillus polymyxa, Streptomyces spp.*	2	5	*Bacillus sp.*, 98%**	*Bacillus subtilis*, 98%, *Bacillus firmus*, 97%, *Bacillus vallismortis*, 98%, *Bacillus cereus*, 98%
Myco tea	IIB	Nutri-Tech Solutions (Australia)	*Azotobacter chroococcum, Bacillus polymyxa*	2	5	-	*Bacillus subtilis*, 97%, *Stenotrophomonas sp.*, 98%, *Ochrobactrum intermedium*, 99%, *Cellulosimicrobium sp.*, 97%, *Bacillus sp.*, 98%,
PHC Biopak	IIB	Plant Health Care (USA)	*B. licheniformis, B. subtilis, B. megaterium, B. polymyxa, B. thuringiensis, Paenibacillus azotofixans*	6	4	*Bacillus subtilis*, 98%, *Bacillus megaterium*, 97%, *Bacillus licheniformis*, 98%	*Bacillus cereus*, 96%
PHC Colonize AG	IIB	Plant Health Care (USA)	*Paenibacillus azotofixans, Bacillus licheniformis, B. megaterium, B. polymyxa, B. subtilis, B. thuringiensis*	6	3	*Paenibacillus sp.*, 97%, *Bacillus subtilis*, 97%	*Bacillus cereus*, 96%
PHC Complete plus	IIB	Plant Health Care (USA)	*Bacillus azotofixans, B. licheniformis, B. subtilis, B. megaterium, B. polymyxa, B. thuringiensis Streptomyces griseoviridis*	7	8	*Bacillus thuringiensis*, 96%, 2 *Bacillus spp.*, 97%**	*Lysinibacillus fusiformis*, 97%, *Peanibacillus alvei*, 97%, 2 *Lysinibacillus spp.*, 97%, *
Fertosolflo	-	SoyGro (South Africa)	No information	-	2	*Bacillus sp.*, 96%, *	
Nemablok	-	SoyGro (South Africa)	No information	-	4	*Bacillus sp.*, 97%, *Burkholderia cepacia*, 98%, *	

*: remaining strains are under sequencing
**: the species have not been determined

The results show that for half of the products (13 of 27), more strains than expected have been isolated. This could denote:

1. A remarkable level of contamination and this is regardless of the content of the products (about 50% of all the products in every category).
2. The companies producing the products involved in this study do not give the entire information on the exact composition of the products and thus the strains are not obviously “contaminants”.

Generally, in the products supposed to contain a large number of bacterial species, less strains than expected have been isolated. This demonstrates the incapacity of too many bacteria to be present and active within a single product. For example, Myco Apply soluble maxx is supposed to contain 15 strains but only 7 strains were isolated (less than 50%), same for PHC colonize which claims to contain 6 strains but only 3 strains were isolated.

Sequencing results show that only 33% of the products contain the expected strain(s) (most likely only one type of bacteria such as *Bradyrhizobium* or *Bacillus*) without any contamination and thus can be considered as “pure products”. On the other hand, products supposed to contain many types of bacteria are mainly highly contaminated (*Bacillus, Enterobacter, Brevibacillus, Paenibacillus*...) and only some of the expected strains are isolated. These “contaminated products” represent about 44% of the products. Eventually, about 15% of the products contain none of the expected strains but only contaminations.

5. Conclusion

- Presence of a variety of strains with different functions (N-fixation, disease suppression, nutrient uptake…) contained in the products.
- Many private companies commercialize a mix of non-defined products and thus do not ensure the quality of their inoculums. This may explain their lack of impact on the plant growth under greenhouse conditions.
- Companies do not provide sufficient information regarding the exact composition of the products.
- Global high level of contamination: “Pure products”: represent 33% of the products only and 41% of the products contained all the expected strains (plus some others). 52% of the products contain none or part of the expected strains and are highly contaminated.

5. References

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